BOTANICAL GAZETTE

NOVEMBER, 1901

NEW OR LITTLE KNOWN UNICELLULAR ALGAE.

II.—EREMOSPHAERA VIRIDIS AND EXCENTROSPHAERA.

GEORGE THOMAS MOORE.

(WITH PLATES X–XII)

Eremosphaera viridis.

AMONG the many algae which have been supposed to represent stages in the life history of other plants, perhaps none have had such a variety of positions ascribed to it as the beautiful spherical form known as *Eremosphaera viridis*. This species was described by De Bary in 1858 in his *Untersuchungen über die Familie der Conjugaten* as a desmid, although he had not observed anything resembling conjugation in the plant, and could only determine its affinities by the mode of division and its general similarity to other well known desmid forms. About the same time, Henfrey (5) found this unicellular organism in bogs in Northumberland, and described it as *Chlorosphaera Oliveri*. A good account of the general structure of the plant is given, and because of certain appearances which he assumed to be antheridia, Henfrey says "in the vicinity of Oedogonieae they (Chlorosphaera) will find their true place." Hofmeister (6), who described Eremosphaera in his memoir on the Desmidiaceae and Diatomaceae, without giving it a name, regarded it as a link between the Desmidiaceae and Palmelleae.

Twenty-five years later De Wildeman in a measure confirmed the view of De Bary, but stated that Eremosphaera

more probably belongs to some developmental stage of a desmid, possibly a zygospore, than represents the vegetative condition of any unicellular alga. More recently De Toni (3) describes the genus as an exceedingly doubtful one, suggesting that the large cells resemble fern prothallia, and that we may expect to find it related to the higher cryptogams. Wille (7), in Engler and Prantl, places Eremosphaera among the Pleurococcaceae. The last investigator to have had this alga under consideration is Chodat (1), who believes that its affinities are such as to put it with the Volvocaceae; furthermore, the results of his observation show a condition of polymorphism which has not hitherto been suggested. In addition to the regular vegetative condition and the ordinary methods of multiplication, which will be described later, Chodat found a number of transition stages which he variously designated as "Gloeocystis" forms, "Palmella" forms, "Schizochlamys" forms, "Centrosphaera" forms, etc. According to Chodat, these various stages of Eremosphaera are actually the same thing as the genera which they resemble, and we can no longer recognize them as distinct species, but must include them all under the single name *Eremosphaera viridis*. It was with the desire to clear up the question as to the polymorphism of this form, and to settle, if possible, its life history and affinities, that the investigation concerning this alga was undertaken.

MATERIAL.

The specimens first studied were secured from a small pool formed by a sluggish brook running through a marsh near Ridge hill, Mass. Although the pool is quite small, there is usually plenty of water, and at no time during the year is the material absent from this locality. There was no sphagnum growing in the pool, as seems usually to be the case where Eremosphaera is found, but an abundance of Zygnema, Spirogyra, and other Conjugatae, notably Micrasterias, was frequently present. The plants were first discovered in May 1897, when they numbered about twenty to the cubic centimeter. Since that time they

have been collected during almost every month of the year, always in considerable quantities.

In August, 1899, while on Naushon, one of the Elizabeth islands, near the place on the east shore known as Tarpaulin cove, Eremosphaera was found in its typical habitat, namely a low sphagnum swamp. There was practically no difference between the forms from the two localities except that of size, and this was quite characteristic. Out of the two hundred and fifty measurements of the specimens from Ridge hill (*fig. 1*), the average diameter was 75.45μ with a maximum of 105μ and a minimum of 67.5μ; while of the same number of spheres from Naushon (*fig. 3*) the average diameter was but 35μ with a minimum of 31.5μ and a maximum of 40.7μ. The figures above referred to will give a fair idea of the comparative sizes of the two lots of material.

This marked difference in size has been noticed by Chodat (1), who in the spring of 1892 found Eremosphaera which was almost exclusively the large form, while in 1894 he discovered among Sphagnum and Carex a quantity of the small variety. The relative sizes of these two extremes is not given, except for the statement that by careful search one might find "giant individuals" of 170μ. De Bary, in his original notice of the plant, gave the measurements as about 60μ, while Rabenhorst says 43–49μ. Kischner gives 100–145μ, and De Toni covers both extremes by citing 100–150μ, as the usual size, 30–80μ specimens being occasionally found. It is evident, then, that the plant is one varying within wide limits. The maximum of the Naushon form not coming within 26μ of the minimum of the Ridge hill form, if size be a sufficient criterion there would be no difficulty in recognizing a variety *minor*, measuring from 30–41μ, and a variety *major*, measuring from 67–100 or more microns. There can be no question that the smaller forms, found by me at Tarpaulin cove, were mature plants. The arrangement of the chromatophores and general cell contents was in every way identical with the larger forms, and division took place as readily in cells 33μ in diameter as in those measuring 100μ. Furthermore,

cultures grown from material from the two localities have retained their characteristic size through a cultivation of more than eighteen months in one case, and for over three years in the other.

GENERAL STRUCTURE.

In appearance Eremosphaera resembles a perfect gelatinous sphere with numerous minute chromatophores usually lining the wall. The arrangement of the chromataphores varies greatly, hardly any two specimens showing exactly the same pattern. Usually they are scattered about in an irregular fashion, sometimes singly, sometimes in groups (*fig.* 1); or they may radiate from the center in a quite definite manner (*fig.* 10). That light has much to do with the arrangement of the color-bodies may be seen from *fig.* 4. This specimen while kept in subdued light showed a cell of almost solid green hue, so thickly were the chromataphores lining the wall. After five minutes in bright sunlight, however, the condition shown in *fig.* 4 was obtained, and in watching the effect under the microscope the chromatophores could be seen sliding along the protoplasmic strands which radiated from the centrally placed nucleus.

When the chromatophores are at the periphery of the sphere and are not too great in number, the nucleus is easily recognized without the aid of stains. The method recommended by Chodat (1) of clearing with chloral solution and staining with carmine brings it out well, and also reveals one or more nucleoli (*fig.* 2). At times the nucleus and the protoplasmic mass surrounding it become quite granular, giving the appearance of some foreign body within the cell. The strands which radiate from the nucleus connect with the protoplasm lining the cell wall and form quite a complete network (*fig.* 5).

The chromatophores are irregular both as to outline and as to size, varying from circular, through broadly elliptical, to narrow fusiform (*fig.* 16). Occasionally they are angular, of a rhomboidal outline, a type especially common in the variety *minor*. The honeycomb appearance described by Chodat (1)

was not visible in my material, neither was the platelike expansion which he says is drawn out from the middle of the chromatophore and may consist of two or three bent wings, causing an irregularly shaped dark spot in its center. A good sized pyrenoid is always distinctly visible, there being sometimes as many as three or four in each chromatophore. If the chromatophore be treated after the manner of Mayer, first adding a dilute iodine solution and then chloral hydrate, the crystalline structure of the pyrenoid is easily seen (*fig. 18*), and the layer of starch surrounding it made visible.

The wall of Eremosphaera is normally thin and of but a single layer in thickness, but it has the property of great gelatinization, and is often laid down in successive layers. This condition gives rise to the forms characterized by Chodat as the Gloeocystis, Palmella, and Schizochlamys stages. The duplication of the wall does not seem to be confined to particular periods of development, but may occur at any time. Each wall soon becomes separate and distinct from the adjoining ones, and with care the several coats may be broken one at a time and the contents allowed to slip out. That such forms are really what botanists recognize as Gloeocystis or Schizochlamys is doubtful. While it is possible that Emerosphaera, surrounded by a number of gelatinous walls, might be mistaken by a hasty observer for a similar condition in either Gloeocystis or Schizochlamys, it does not necessarily follow that these genera are one and the same thing. Schizochlamys, I have been able to cultivate for a considerable length of time, and it has never exhibited any evidence of being related to Eremosphaera. Since we can have the same duplication of the wall in Chlamydomonas and other genera widely separated from the ones under discussion, it hardly seems a sufficient basis upon which to unite a number of forms frequently showing marked differences in their life histories.

In addition to the successive formation of coats around Eremosphaera, there sometimes occur peculiar growths on the inner surface of the wall, which are the result of a number of layers of cellulose being formed about a central point (*fig. 11*).

These concentric layers can occasionally be separated by crushing, but usually they are very compact. They project inward towards the center of the cell, and in case a second continuous wall is formed, it remains indented wherever these excrescences appear. As many as twelve secondary walls have been counted around a single sphere, and there seems to be no limit to their formation.

CULTURE METHODS.

A plant which has had as many developmental stages ascribed to it as Eremosphaera naturally necessitates the most careful application of pure culture methods. Consequently the cultivation of this plant was attempted as soon as it was procured, and cultures have been kept running successfully for about three years. As it was not convenient to visit the original locality frequently, and to obtain fresh material in that way, it seemed best to maintain a number of gross cultures from which pure transfers might be made at any time. No trouble was experienced in this, and water from the original pool, containing diatoms, numerous filamentous algae, and Eremosphaera, has been kept continuously in the laboratory. Bacteria appeared for a short time, but the water soon cleared, and all the algae have maintained themselves in good condition. These gross cultures were kept either in crystallizing dishes or wide-mouthed bottles, over which was a sheet of glass to prevent too rapid evaporation. It was found that the crystallizing dish was most convenient for this purpose because of the ease with which material could be picked out from it. The large Eremosphaera cells were readily found with a hand lens, and then transferred by means of a pipette to a watch glass. Here they were washed several times in sterilized water, and then examined under the microscope, before being placed on the culture medium.

Various methods of cultivation were tried. The well-known solution of Knop was used in strengths from 0.2 per cent. up to 1 per cent., both as a fluid culture and in connection with agar agar and gelatin. The gelatin was soon abandoned, however, on account of the low temperature at which it liquefied, since

agar agar (5 to 7 grams to the liter of nutrient solution) enabled one to keep perfect trace of the cells, and was more satisfactory for many reasons. Stender dishes were used for culture vessels because it was desirable to have as large an amount of the medium as possible, so that the cultures would last for some time, and in order that there might be a considerable surface for growth. The same precautions of sterilization were observed that would have been necessary for bacteriological work, and check cultures indicated that these methods were successful.

Usually after sterilization there would be a considerable amount of moisture collected upon the surface of the agar, and it was in this fluid that the Eremosphaera cells were deposited. If placed directly upon the surface of the agar it seemed impossible for them to persist, but when transferred to the moisture and then gradually, through the evaporation of the superfluous liquid, brought in contact with the nutrient agar there was no difficulty in making them grow as well as in fluid media.

Van Tieghem cell methods were not successful. The plants lived for a considerable length of time and the chlorophyll granules moved under the influence of sunlight, but there was no development of any kind. Cultures kept in tightly sealed cells for several months would gradually present an appearance resembling the formation of spores, and at first this was thought to be the case. Upon investigation, however, it was found that this effect was due entirely to the rounding off of the chromatophores, which were closely packed at the periphery of the sphere. Upon being restored to natural conditions such cells resumed the characteristic arrangement of the color bodies, and the nucleus at no time presented any other appearance than that found in the normal plant.

Ward cells, which allowed a constant supply of air and yet prevented any contamination from the outside, were partially successful. The most satisfactory method for making direct observations of cultures, however, was to isolate a single individual and place it on a slide either in sterilized water or a nutrient solution. A large cover-glass was then placed over it, being

propped up by three or four minute drops of a mixture of beeswax and vaseline. A bell jar lined with moist filter paper was sufficient to prevent the evaporation of the solution for twenty-four hours, and a drop of nutrient fluid added at one side of the cover-glass replaced any loss during the examination under the microscope. Such cultures require daily examination, of course, but when the life history of an organism is being followed out this is an advantage rather than an objection. The wax supports for the cover-glass were preferable to bits of glass or filter paper because they permitted the crushing out of a specimen, if necessary, and held the cell in just the desired position. Cultures of this kind gave the best of results, the algae apparently developing in a perfectly normal manner.

MULTIPLICATON AND DEVELOPMENT.

De Bary, in his observations upon Eremosphaera, discovered that it multiplied by simple division into two or four cells, each of which soon attained the size of the original plant. Henfrey likewise recognized this condition. Such a division is accomplished by the formation of a new wall between the two halves of the original cell. This wall extends completely around each half, and as the nucleus divides and the chromatophores, with the protoplasm, form into two irregular masses, the gelatinous wall pushes in from the periphery, forming the division (*fig. 6*). The two cells thus formed gradually increase in size, so that in a short time the original wall is ruptured and the daughter plants are liberated. These usually escape as perfect spheres, but may retain their flattened appearance for a short time, as is shown in *fig. 7*. More rarely a second division may take place at right angles to the first, thus producing four daughter cells instead of two (*fig. 8*). Wolle (8) and Chodat (1) both speak of even further successive divisions, but my material has never shown more than four cells formed in this way.

This method of simple division has been believed to be the only means of propagation in Eremosphaera, and it was not until the result of the researches of Chodat became known, that

a more complicated life history, including the formation of zoospores, was suspected. Henfrey, in his discussion of the plant already referred to, suggested the possibility of antherozoids, but this condition was undoubtedly due to the presence of some parasite, which Henfrey himself recognized as a possible cause.

While considerable doubt has been cast upon Chodat's interpretation of what he saw, the fact that certain of the stages which he describes certainly exist in material found in this country, makes it necessary that a most careful search be made for all the forms supposed by him to be connected with Eremosphaera. In addition to the well-known division into two or four spheres, Chodat (1) describes a kind of sporangium-building condition where division goes on until the diameter of the ultimate cells is ten to twenty times smaller than at first. This stage I have been unable to find, and nothing of the sort seems to occur in the material I studied, either in gross or pure cultures. In addition to these non-motile spores, Chodat describes ciliated spores. Just how and when these may be formed is not clear, but it is to be inferred that they arise from some "palmella" condition and not directly from the adult plant. These zoospores, according to Chodat, are usually elliptical, and are always surrounded by a gelatinous coat, reminding one of certain forms of Chlamydomonas. Each zoospore has a red eye spot, a radiating chromatophore, and an evident nucleus. In most cases there are two cilia, but exceptionally three. The question as to whether they actually had four remained undecided, as did also the possibility of their conjugation. They seem to have been irregular both as to size and shape, for Chodat (1) says, "da ihre Mutterzellen an Grosse sehr verschieden sein konnen, so sind infolgedessen die Zoosporen auch sehr verschieden, sowohl was die Grosse als auch die Form anbetrifft."

Although a most careful search has been made among material growing under all conditions, not the slightest trace has ever been discovered of any motile spore in evident relation to Eremosphaera. Occasionally plants would be found of a lighter

green color, filled with spherical bodies (*fig. 13*), which in general resembled a sporangium. But while these cells were observed daily for weeks they not only failed to show any signs of further development, but gradually disintegrated instead. After having observed algae which were crushed and noting the manner in which the chromatophores formed a colorless membrane about themselves, with the green collecting at one side, as in *fig. 17*, it would seem almost certain that the conditions I had been observing were due to injury, and the admission of a small amount of water caused the abnormal appearance suggesting spores. On one occasion, a plant of this sort showed four or five biciliate organisms within it, which at first were supposed to be zoospores (*fig. 14*). Their subsequent development (*fig. 15*), however, proved them to have no connection with the alga, and after three days, when the culture was crushed out, it was found that the organisms possessed no chlorophyll, a fact which could not be made clear with certainty while they were embedded among the chromatophores.

Failing to find zoospores under natural conditions, it was hoped that some change in the environment might be produced which would cause zoospore formation. Plants, closely encysted in numerous gelatinous coats and which had been growing in a nutrient solution, were removed to fresh water and to variously modified media. Some cultures were allowed to remain in the sunlight, others were kept in darkness. Specimens taken direct from nature were placed in numerous solutions and subjected to all possible changes of temperature and moisture. Aeration was tried for lengths of time varying from a few hours to several weeks, and cells were kept on ice over night, and then gradually brought into the temperature of the laboratory. But none of these methods yielded any thing more than the previously described method of division. Consequently, after having had cultures running for over three years, and making repeated examinations of material collected under natural conditions, it must be said that at no time was there any appearance which gave the slightest evidence of zoospore formation, and it

is certain that in the forms found growing in this country zoospores must be very rarely, if ever, formed.

The rejuvenescence described by Chodat (1), wherein a cell instead of dividing simply slips out from the old sheath, was frequently seen and is represented in *fig. 5*. Whether this can really be termed a rejuvenescence, according to the ordinary use of the term, is a question. It seems as though this condition can hardly be different from those which show a succession of walls, except that in the first case the outer wall is not strong enough to hold the cell, and it slips out as indicated. There never appears to be any change in the contents of the cell, not even in the arrangement of the chromatophores.

In the mud at the bottom of the water containing Eremosphaera, there will usually be found a considerable number of resting cells. These are of the typical brick-red color, and generally show successive layers of sheaths. They contain large quantities of oil, and in no way appear to differ from the same type of spore in other algae. So far as observed, the development of the resting spore was not accompanied by the formation of any new cells. The red color was gradually lost, the chromatophores became more and more prominent, and the normal vegetative appearance of Eremosphaera was the result. Usually the resulting cell is quite large and division takes place in a very short time. No division of the resting spores was observed, the condition indicated in *fig. 19* being an example of where two cells passed into the resting condition almost immediately after division, and before they had time to enlarge. This is not an infrequent occurrence.

TAXONOMIC POSITION.

It is evident from the foregoing that there is no reason for supposing that Eremosphaera is related to the desmids or to any of the Conjugatae; neither does it seem possible to show that it forms any part of the life history of any of the higher cryptogams. The theories of Henfrey (5) and Hofmeister (6) may also be dismissed, so that there only remain the two positions

ascribed to it by Chodat (1) and Wille (7). Chodat, as the result of his study of Eremosphaera, came to the conclusion that it had evident affinities with the Volvocaceae, and if all the stages described by him are really passed through by this plant, it would seem as though some such disposition would have to be made of it. On the other hand, the negative results of my cultures and observations would seem to throw some doubt upon its supposed polymorphism, particularly with regard to a motile stage. It seems, therefore, that for the present at least, Eremosphaera should be classed with the group of green algae possessing no zoospores, namely the Protococcoideae.

Excentrosphaera, nov. gen.

There still remains to be discussed the form described by Chodat as the "Centrosphaera" stage. This form was present in small quantities in the first material collected at Ridge hill, and was immediately separated from the Eremosphaera cells and cultivated. The growth was luxurious in fluid nutrient media, and satisfactory, if not so abundant, on agar. In addition to these pure cultures, a number of dishes containing water from the pool which supplied the original material were kept in the laboratory, and in the course of a few months became filled with a large number of these plants. The conditions for growth under these various circumstances seemed favorable and normal, but there was at no time any evidence of the slightest connection with the developmental history of Eremosphaera. After having had this form under cultivation for about three years, and having seen it pass through its life cycle over and over without assuming a stage which in any way resembled those of Eremosphaera, I am obliged to conclude that it is an independent genus. Since it does not seem to have been described elsewhere, the name *Excentrosphaera viridis* has been given to this alga.

Excentrosphaera, in its mature condition, may assume an outline varying from that of a perfect circle, through all gradations to an ellipse, as well as occasional excentric and indefinite shapes. The general resemblance to the forms assumed by germinating

Ulothrix spores is striking, but the much larger size, the arrangement of the chromatophores, and suosequent development make it an easy matter to differentiate this plant from that genus.

The numerous large chromatophores are crowded near the surface of the cell, and are usually arranged radially (*figs. 21, 23, 24*). As the cells assume irregular shapes the chromatophores may be distributed in various ways, and there are often lacunae between the chlorophyll masses. Sometimes there are several layers of chromatophores, so that except for the nucleus and a small amount of protoplasm, the cell is almost filled with these bodies. There are numerous minute pyrenoids in each chromatophore, but these are not readily made out without the use of stains. The densely packed chromatophores render it difficult to see the centrally placed nucleus, with its nucleolus, but sections (*fig. 25*) or crushing will usually reveal it. When the cells are about to form spores the nucleus divides repeatedly (*fig. 26*), then the chromatophores break up, and in a short time the entire cell has a homogeneous appearance similar to that shown in *fig. 27*. The spores, which are formed from this condition simultaneously, are 2–3μ in diameter, non-motile, and without a red spot. They escape through a hole formed by the dissolution of the wall (*fig. 22*), and in about a month increase to the size of the mature plants. The developing spore usually remains spherical until it has reached its maximum size, and not until then does it begin to take on the irregular shape previously referred to.

The wartlike projections on the wall, reported by Chodat (1), frequently occur in plants after maturity has been reached, but in small spherical forms are quite rare. These formations are made up of a series of layers of cellulose, and often increase until they are of considerable size (*figs. 23, 24*).

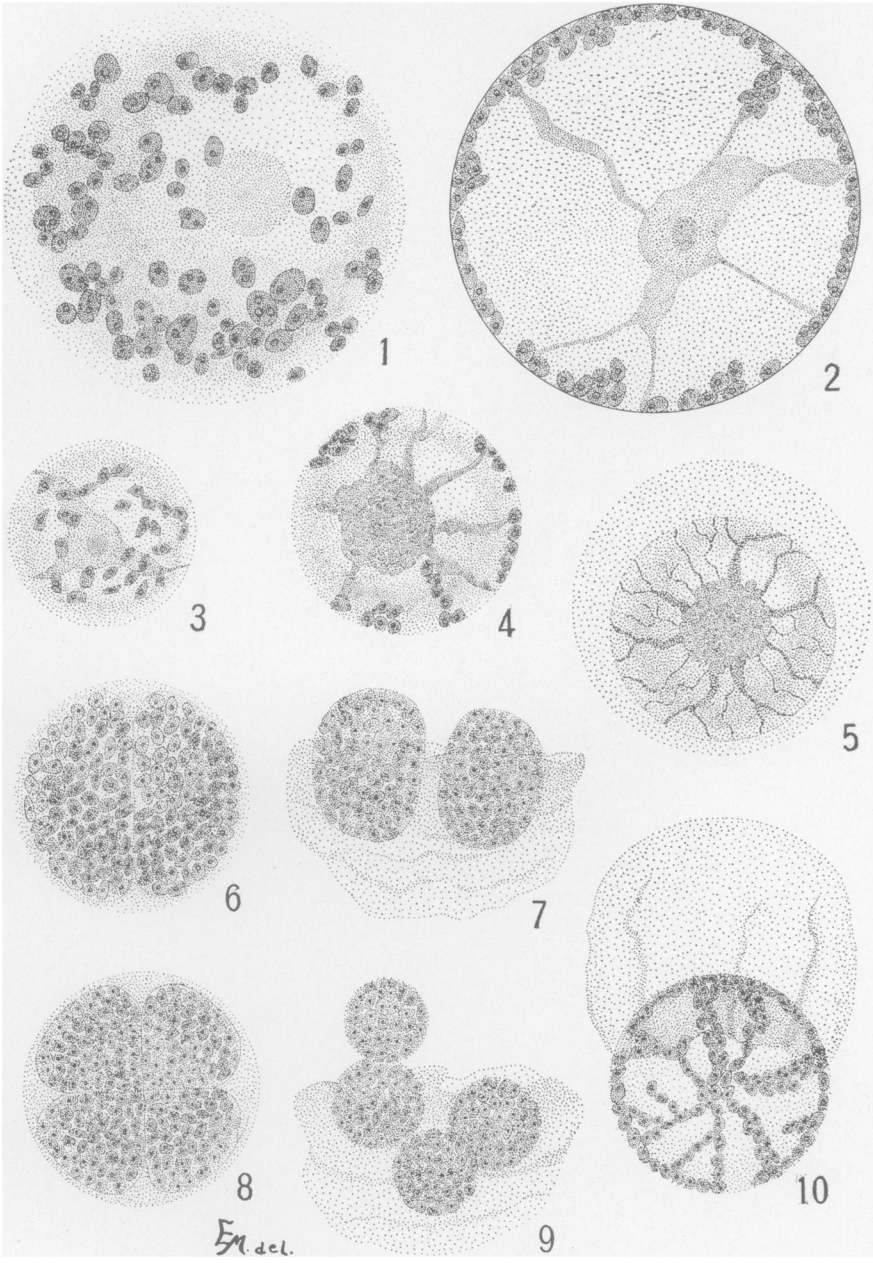
Resting conditions, with a very thick wall and of a reddish color, were found in pure cultures, but the mode of development has not been observed up to the present time. As previously stated, this plant has been cultivated in a pure state for several years, and all the methods resorted to in an effort to bring about

zoospore formation in Eremosphaera were repeated with Excentrosphaera, but without success.

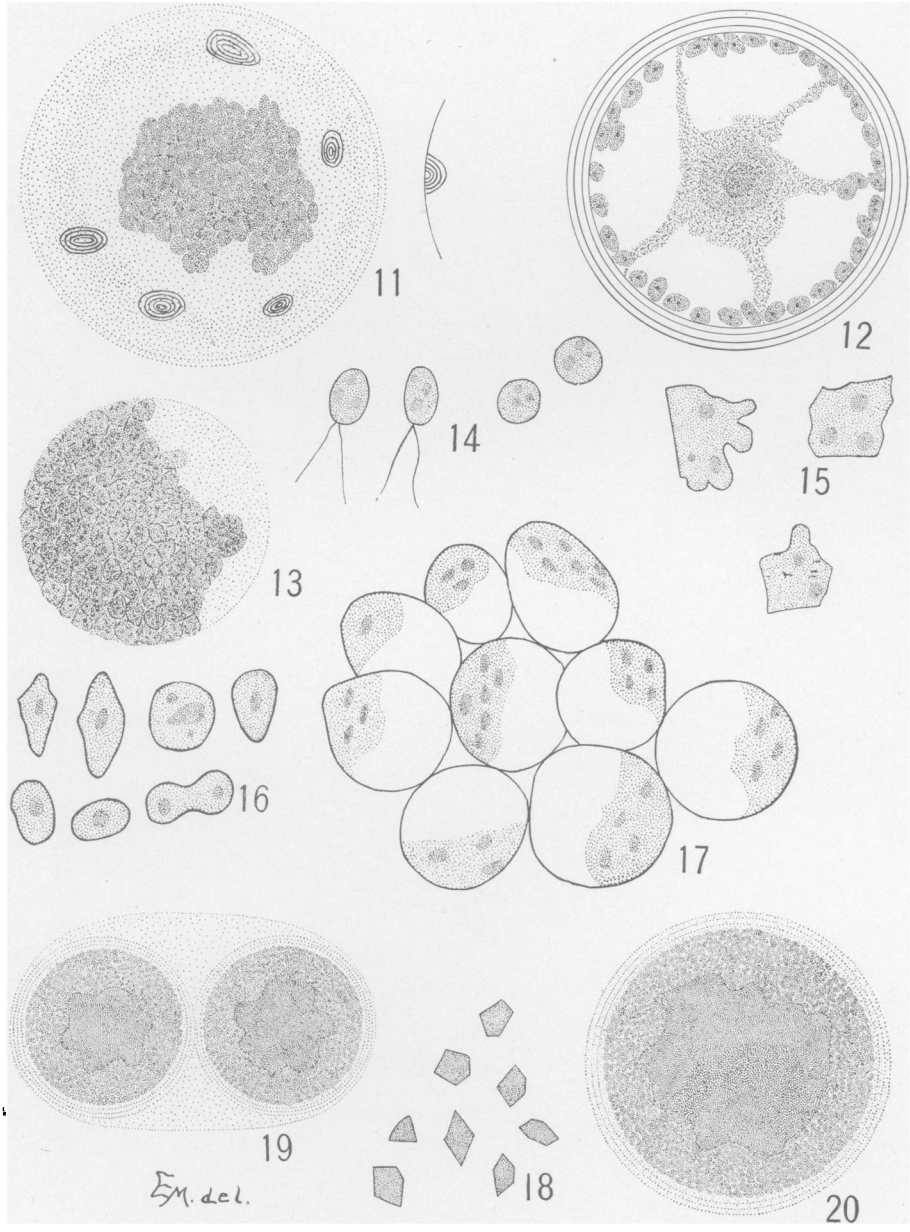
The Eremosphaera material found on Naushon has never shown Excentrosphaera, nor has the Ridge hill material developed it after the original lot was collected. Excentrosphaera has been found in stagnant ponds near Norwich, Vt., which contained, in addition, large amounts of Nitella, Spirogyra, Oedogonium, and related forms. A shallow pool, almost filled with Hydrodictyon, not far from Boston, has also furnished Excentrosphaera in considerable quantities. Neither of these latter localities have ever shown Eremosphaera, although repeated search has been made for it. Unless we are to adopt Borzi's "stadii anamorphici" for all the algae, it does not seem possible that this plant has any genetic connection with any other form. The external resemblance to Centrosphaera, which led Chodat to give that name to it as a supposed stage of Eremosphaera, is certainly striking, but the decided difference in habitat, together with the absence of motile spores and the difference in development, would seem to be sufficient to separate it from that genus. The affinities of Excentrosphaera, so far as known, must be with the Protococcaceae of Wille.

Excentrosphaera, nov. gen.—Plant consisting of a single cell, in mature condition varying in outline from spherical and elliptical to irregular and excentric forms. Chromatophores large, angular, usually radiately arranged, closely lining the wall. Pyrenoids minute, numerous in each chromatophore. Multiplication by means of non-motile spores (aplanospores), which escape by the dissolution of a part of the cell wall. Reaction to all external stimuli negative.

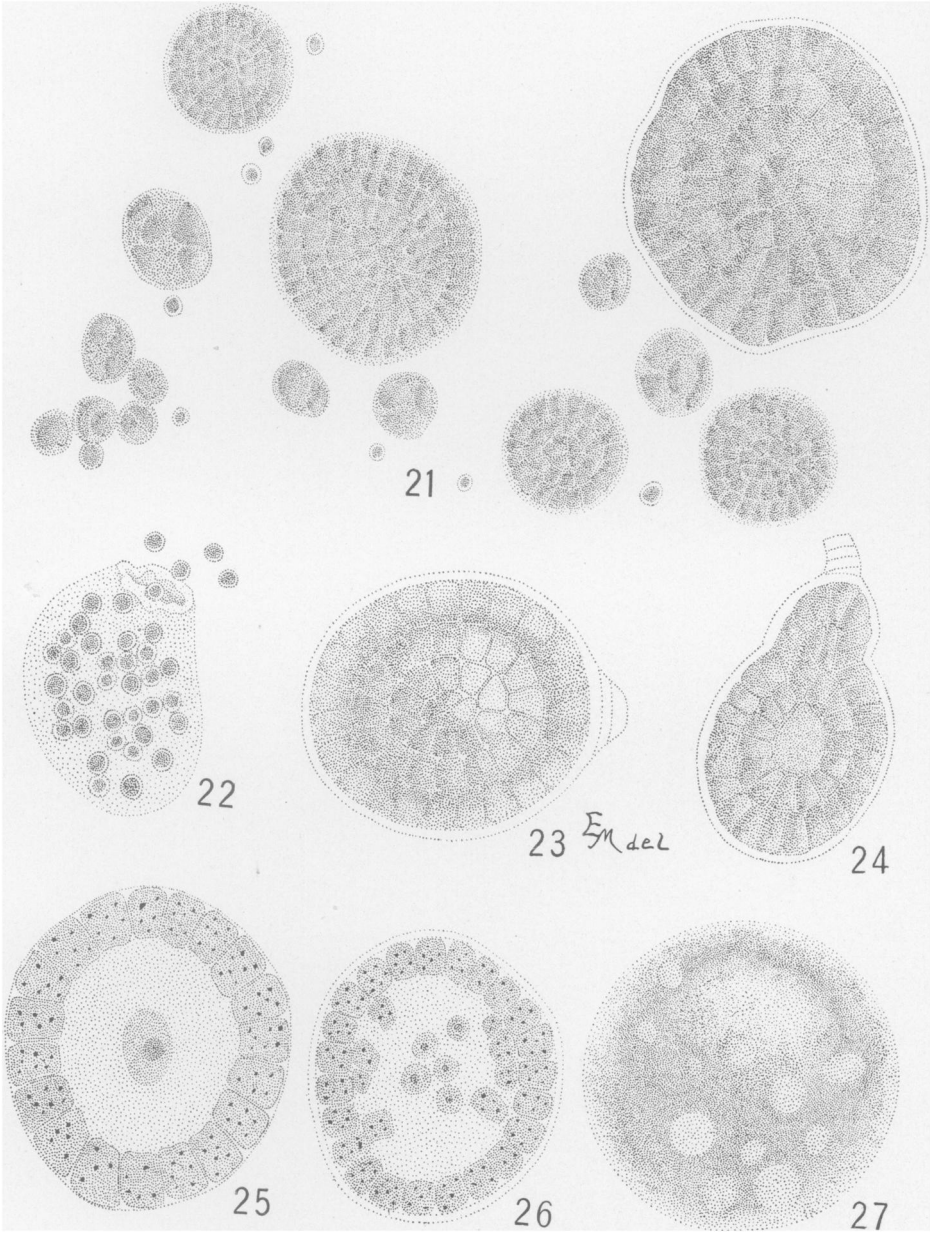
E. viridis, nov. sp.—*Plate XII, figs. 21–27.* Characters of the genus. Plants of bright green color; size of mature cells 22–55μ. Spores 2–3μ. Growing with Eremosphaera, Geneva (?); with Eremosphaera, Micrasterias, Zygnema, etc., Ridge hill, Mass., the year around; in swamps with Nitella and various algae, Norwich, Vt., September–November; with Hydrodictyon in shallow pool, in vicinity of Boston, June–August.



MOORE on EREMOSPHAERA



MOORE on EREMOSPHAERA



MOORE on EXCENTROSPHAERA

This work was commenced in the Cryptogamic Laboratory of Harvard University, and my sincere thanks are due to Dr. Farlow and to Dr. Thaxter for their helpful criticism of the investigation carried on while there.

DARTMOUTH COLLEGE,
Hanover, N. H.

BIBLIOGRAPHY.

1. CHODAT, R., Ueber die Entwickelung der *Eremosphaera viridis* DeBy. Bot. Zeit. **53**: 137–148. 1895.

2. DE BARY, A., Untersuchungen über die Familie der Conjugaten. Leipzig, 1858.

3. DE TONI, J. B., Sylloge Algarum **1**: 616. 1889.

4. DE WILDEMAN, E., Comptes rendus de la Soc. Roy. de Bot. Belge. 1894.

5. HENFREY, A., On Chlorosphaera. Trans. Micr. Soc. Lond. **7**: 25–29. 1859.

6. HOFMEISTER, W. F. B., Ueber die Fortpflanzung der Desmidieen und Diatomeen. Leipzig. 1857.

7. WILLE, N., Die natürlichen Pflanzenfamilien, Algen **1**[1]: 58. 1897.

8. WOLLE, F., Freshwater algae of the United States. **1**: 201. 1887.

EXPLANATION OF PLATES.

All the figures are from ink drawings sketched in with an Abbé camera. In the reproduction they are reduced about one fourth. *Figures 14–18* are drawn with a Leitz $\frac{1}{12}$ (oil), oc. 3; all the others with a Leitz $\frac{1}{5}$ oc. 3. The magnifications given are the original ones before reduction and allow for projection.

PLATE X. Eremosphaera viridis De Bary.

FIG. 1. Surface view of large variety (Ridge hill material). × 250.

FIG. 2. Section of same showing nucleus and protoplasmic strands. × 250.

FIG. 3. Surface view of small variety (Naushon material). × 250.

FIG. 4. Surface view showing the retreat of the chromatophores under the influence of strong sunlight. × 250.

FIG. 5. So-called "rejuvenescence." × 250.

FIG. 6. Beginning of the division into two. × 250.

FIG. 7. Division completed and liberation of daughter cells. × 250.

FIG. 8. Division of mother cell into four. × 250.

FIG. 9. Division completed and liberation of daughter cells. × 250.

FIG. 10. Escape of cell from old wall after "rejuvenescence." × 250.

PLATE XI. Eremosphaera viridis De Bary.

FIG. 11. Surface view and section of wartlike formations in wall. × 250.

FIG. 12. Section showing successive formation of walls. × 250.

FIG. 13. Abnormal condition resembling zoosporangium. × 250.

FIG. 14. Foreign organisms found within cell similar to *fig. 13*. × 830.

FIG. 15. Ultimate development of ciliated organisms. × 830.

FIG. 16. Normal appearance of chromatophores, with pyrenoids. ×830.

FIG. 17. Chromatophores after being crushed out in water. × 830.

FIG. 18. Pyrenoids after treating with chloral hydrate. × 830.

FIG. 19. Resting spores formed immediately after division. × 250.

FIG. 20. Resting spore formed from mature plant. × 250.

PLATE XII. Excentrosphaera viridis Moore.

FIG. 21. General appearance with successive stages in development from spore. × 250.

FIG. 22. Escape of spores. × 250.

FIGS. 23, 24. Cellulose projections of wall. × 250.

FIG. 25. Section through cell, stained to show pyrenoids. × 250.

FIG. 26. Section showing first divisions of nucleus previous to spore formation. × 250.

FIG. 27. Homogeneous appearance of cell previous to spore formation. × 250.